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Claims

1. An encoded beaded ~~or granulated~~ polymer matrix comprising a plurality of spatially immobilised particles ~~or vacuoles~~, wherein each particle or vacuole is individually detectable, wherein the encoded bead has a diameter of from 0.5 millimeter to less than 2.0 millimeter and comprises from 3 to 10 microparticles.

2. The beaded or granulated polymer matrix according to claim 1 in which the matrix has different optical or spectroscopic properties from those of the immobilised particles or vacuoles.

3. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles or vacuoles are monodisperse.

4. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are heterodisperse.

5. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are spherical.

6. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are irregular particles.

7. The beaded or granulated polymer matrix according to claim 1 comprising from 4 to 10 particles.

8. The beaded or granulated polymer matrix according to claim 1 comprising from 4 to 8 particles.

9. The beaded or granulated polymer matrix according to claim 1 comprising less than 5 particles.

10. The beaded or granulated polymer matrix according to any of claims 7 to 9 being essentially spherical.

11. The beaded or granulated polymer matrix according to any of claims 7 to 10, wherein at least two of said particles have essentially the same diameter.

12. The beaded or granulated polymer matrix according to any of claims 10 and 11, wherein all of said particles are essentially monodisperse.

13. The beaded or granulated polymer matrix according to any of claims 7 to 12, wherein said particles are less than 10 micrometer in diameter.

14. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 5 micrometer in diameter.

15. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 1 micrometer in diameter.

16. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 0.1 micrometer in diameter.

17. The beaded or granulated polymer matrix according to any of claims 7 to 16, wherein said particles comprise a spectroscopically detectable marker.

18. The beaded or granulated polymer matrix according to any of claims 7 to 16, wherein said particles comprise a fluorescently detectable marker.

19. The beaded or granulated polymer matrix according to claim 18, wherein the fluorescently detectable marker is selected from the group consisting of dyes based on the structure of fluorescein, oregon green, rhodamine, aminobenzoic acid, Alexa™ probes, BODIPY-dyes, cascade blue dye, coumarine, naphthalenes, dansyl, indoles, pyrenes pyridyloxazole, cascade yellow dye, Dapoxyl Dye, Fluorescamine, aromatic ortho dialdehydes, OPA and NDA, ATTO-Tag's, 7-Nitrobenz-2-Oxa-1,3-Diazole or derivatives thereof,

20. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is selected from the fluorescent group of

compounds and materials consisting of fluorencent organic polycyclic compounds, conjugated vinylic compounds, heterocyclic transition metal complexes, rare earth metal compounds, inorganic oxides and glasses.

21. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by two photon fluorescence spectroscopy.

22. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by one photon fluorescence spectroscopy.

23. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by time-correlated photon fluorescence spectroscopy.

24. The beaded or granulated polymer matrix according to claim 1, wherein the polymer is optically transparent in the optical excitation of said fluorescent marker and/or the emission wavelength ranges of said fluorescent marker.

25. The beaded or granulated polymer matrix according to claim 1, wherein the polymer is selected from the group consisting of polyethers, polyvinyls, polyacrylates polyacylamides polyacrylamides, polystyrenes, polycarbonates, polyesters, polyamides, and combinations threreof.

26. The beaded or granulated polymer matrix according to claim 1, wherein the polymer is selected from the group consisting of SPOCC, PEGA, HYDRA, POEPOP, PEG-polyacrylate copolymers, polyether-polyamine copolymers, crosslinked polyethylene diamines, and combinations thereof.

27. The beaded or granulated polymer matrix according to claim 1, wherein said particles comprise a marker, which is detectable by fast spectroscopic techniques other than fluorescence spectroscopy.

28. The beaded or granulated polymer matrix according to claim 27, wherein said fast spectroscopic technique comprise Infrared spectroscopy, raman spectroscopy, visble light spectroscopy, UV spectroscopy, electron spin resonance, and nuclear magnetic resonance.

29. The beaded or granulated polymer matrix according to claim 1, wherein said particles comprise a marker, which is detectable by fast detection techniques other than spectroscopy such as light scattering, reflection, diffraction or light rotation.

30. The beaded or granulated polymer matrix according to any of claims 17 to 23 and 27 to 29, wherein the marker is detected by probing the marker with a range of frequencies differing by less than 10% based on the numerical highest frequency value.

31. The beaded or granulated polymer matrix according to any of claims 17 to 23 and 27 to 29, wherein the marker is detected by probing the marker with one or more predetermined frequencies.

32. A composition comprising a plurality of different, spatially encoded, beads according to any of claims 1 to 31, wherein essentially each bead is individually identifiable.

33. The composition according to claim 32, wherein the composition comprises at least 10^2 individually identifiable beads.

34. The composition according to claim 32, wherein the composition comprises at least 10^3 individually identifiable beads.

35. The composition according to claim 32, wherein the composition comprises at least 10^5 individually identifiable beads.

36. The composition according to claim 32, wherein the composition comprises at least 10^7 individually identifiable beads.

37. The composition according to claim 32, wherein the composition comprises at least 10^9 individually identifiable beads.

38. The composition according to any of claims 32 to 37, wherein each beaded polymer matrix comprises at least one site for functionalisation to which a ligand or a bioactive species can be attached.

39. The composition according to claim 38, wherein the site for functionalisation comprises a reactive group or a scaffold comprising two or more of such reactive groups.

40. The composition according to any of claims 38 and 39 further comprising a plurality of ligands or bioactive species attached to the beaded polymer matrices, wherein different ligands or different bioactive species are attached to different beads, wherein more than about 95% of all of said different beads are individually identifiable.

41. The composition according to any of claims 32 to 40, wherein essentially all of said beads are individually identifiable.

42. The composition according to any of claims 32 to 41, wherein the spatial positions of particles in each bead are defined by sets of coordinates (x,y,z) of particle centers of said particles.

43. A method for the detection of relative positions in space of centers (x,y,z) of immobilized particles of the composition according to any of claims 32 to 42, said method comprising the step of recording of at least two 2D-projections of the particles, said method optionally comprising the further step of determining, on the basis of the relative positions in space of centers (x,y,z) of immobilized particles, the distance matrix for individual beads, or a set of geometrical figures, such as triangles, derivable from the relative positions in space of centers (x,y,z) of the immobilized particles.

44. The method according to claim 43 wherein 3 2D-projections are recorded along 3 orthogonal axis x, y and z to generate 3 sets of 2D-coordinates (y,z),

(x,z) and (x,y), respectively, from which the 3D-coordinates (x,y,z) of particle centers can be derived.

45. The method according to claim 43, wherein a plurality or stack of 2D projections are generated by confocal or focal microscopy to recreate the 3D image matrix of the bead from which the relative particle position (x,y,z) in space can be determined.

46. The method of any of claims 43 to 45 employing at least one focussed scanning laser for detection of relative positions in space of centers (x,y,z) of immobilized particles and laminar fluidics for bead manipulation.

47. The method of claim 46 in which the coordinates x and y of a particle position are determined by fast scanning two orthogonally aligned lasers over two cross sections of the moving bead while the z coordinate is determined by the time of flight of the bead at known flow rates.

48. The method of any of claims 43 and 44 in which the coordinates x and y of a particle position are determined by using a single laser and a rotating mirror that via 2 or 3 geometrically arranged static mirrors reflects the laser beam along 2 or 3 orthogonal axes.

49. A method for generating a beaded or granulated polymer matrix comprising a plurality of spatially immobilised particles according to any of claims 1 to 31, said method comprising the steps of

   a. synthesizing a monomer or macromonomer and a crosslinker for polymerization, and

   b. mixing these with the encoding particles to give an even dispersion of particles in the mixture, and

   c. polymerizing the monomer or macromonomer by either i) suspension polymerisation and/or; ii) inverse suspension polymerisation and/or iii) bulk polymerisation followed by granulation, and/or iv) droplet polymerisation.

50. The method of claim 49, wherein the polymerisation reaction is a radical initiated chain polymerisation reaction.

51. The method of claim 49, wherein the polymerisation reaction is an anion initiated ring opening polymerisation reaction.

52. The method of claim 49, wherein the polymerisation reaction is an cation initiated ring opening polymerisation reaction.

53. A method for distance matrix determination of at least one spatially encoded beaded or granulated matrix comprising a plurality of spatially immobilised particles comprising an optically detectable label, said method comprising the steps of

i) providing at least one beaded or granulated polymer matrix according to any of claims 1 to 31,

ii) providing at least one device for recording and storing at least one image of the at least one bead, said device comprising

a) at least one source of illumination,

b) at least one flow system comprising a flow cell comprising an imaging section,

c) at least one pulse generator,

d) at least one image intensifier,

e) at least one CCD camera,

iii) activating at least one source of illumination,

iv) introducing the at least one encoded bead comprising a plurality of particles into the flow cell comprising an imaging section,

v) recording at least one image of the at least one bead by sending substantially simultaneously a pulse generated by a pulse generator to both a) the at least one image intensifier, and b) the at least one CCD camera capable of recording said at least one image, and

vi) determining for individual beads a distance matrix based on the at least one image obtained for each bead in step v),

wherein the distance matrix for an individual bead is determined by a method comprising the steps of

determining for each particle of the encoded bead the 2D coordinates in the XZ-plane and in the YZ-plane, thereby generating a first set of data and a second set of data,

combining the first set of data and the second set of data and thereby obtaining 3D coordinates for each particle, and

calculating the distance matrix as the full set of distances between particles for which preferably only one set of 3D coordinates is obtained.

54. The method of claim 53, wherein steps iv), v), and vi) are repeated for individual steps entering the flow cell.

55. The method of any of claims 53 and 54 comprising the further step of detecting a bead entering the flow cell by using a photo-sensor.

56. The method of any of claims 53 to 55, wherein the pulse generator is activated by the activation of the photo-sensor, and wherein the photo-sensor is activated by an encoded bead entering the flow cell.

57. The method of any of claims 53 to 56 comprising the further step of storing the at least one image on a data storage medium.

58. The method of any of claims 53 to 57, wherein two CCD cameras and two image intensifiers are employed for recording the at least one image of the at least one bead.

59. The method of any of claims 53 to 58, wherein the source of illumination comprises a continuous wave laser capable of illuminating the imaging section of the flow cell.

60. The method of any of claims 53 to 59, wherein the photo-sensor for detecting the entry of an encoded bead into the imaging section of the flow cell comprises an optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of said photo-sensor, wherein said optical objective of said photo-sensor comprises a fluorescence filter capable of blocking the light of said laser, and wherein said fluorescence filter transmits the fluorescence emission from the particles.

61. The method of any of claims 53 to 60, wherein the at least one CCD camera for recording the at least one image of the at least one encoded bead comprises at least one gated image intensifier for amplifying the fluorescence emission from the encoded bead, and wherein each of said image intensifiers comprises at least one optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of each image intensifier, and wherein each optical objective comprises a fluorescence filter for blocking the light of said laser, and wherein the fluorescence filter transmits the fluorescence emission from the particles.

62. The method of any of claims 53 to 61, wherein a plurality of encoded beads are provided and wherein multiple distance matrices are determined based on individual distances recorded for individual beads.

63. The method of claim 62, wherein a set of multiple distance matrices is determined for a subpopulation of the beads based on more than one set of individual distances recorded for the subpopulation of beads.

64. The method of any of claims 62 and 63, wherein each distance matrix is recorded individually.

65. The method of any of claims 53 to 64, wherein at least one image of each bead is recorded per second.

66. The method of any of claims 53 to 65, wherein a total of more than 4000 beads are recorded per hour, such as more than 5000 beads per hour, for example more than 10000 beads per hour, such as more than 15000 beads per hour, for example more than 20000 beads per hour such as more than 25000 beads per hour, for example more than 30000 beads per hour such as more than 40000 beads per hour, for example more than 50000 beads per hour such as more than 60000 beads per hour, for example more than 70000 beads per hour, such as more than 80000 beads per hour, for example more than 90000 beads per hour such as more than 100000 beads are recorded per hour.

67. The method of any of claims 53 to 66, wherein the optical power of the laser is in the range of from 1 mWatt to preferably less than about 200 mWatt, such as about 10 mWatt, for example about 50 mWatt, such as about 100 mWatt, for example about 150 mWatt.

68. The method of any of claims 53 to 67, wherein the wave length of the laser is in the range of from about 450 nm to preferably less than 700 nm, such as about 500 nm, for example about 600 nm.

69. The method of any of claims 53 to 68, wherein the exposure time of the image intensifiers is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

70. The method of any of claims 53 to 69, wherein the exposure time of the CCD cameras is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

71. The method of any of claims 53 to 70, wherein the response time of the photo-sensor is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

72. The method of any of claims 53 to 71, wherein the flow rate of the beads through the flow cell of the flow system is preferably more than 0.01 meter per second, for example more than 0.1 meter per second, such as more than 1 meter per second.

73. The method of any of claims 53 to 72, wherein the dimensions of the imaging section is preferably less than 1 milliliter, such as less than 0.5 milliliter, for example less than 0.1 milliliter, such as less than 0.05 milliliter, for example less than 0.01 milliliter, such as less than 0.005 milliliter, for example about or less than 0.001 milliliter.

74. The method of any of claims 53 to 73, wherein the flow cell is preferably made from a material which does not absorb the illumination light from the source of illumination and/or the emission light emitted from the particles, preferably quartz or a suitable transparent polymer.

75. The method of any of claims 61 to 74, wherein the size distribution of the beads is in the range of from 0.1 millimeter to preferably less than 2 millimeter, such as about 0.5 millimeter, for example about 1 millimeter, such as about 1.5 millimeter, and independently thereof, wherein the diameter of the paraticles is preferably less than 30 micrometer, for example less than 20 micrometer, such as less than 15 micrometer, for example in the range of from 5 to 15 micrometer.

76. The method of claim 75, wherein the majority of the beads, such as more than 75% of the beads, for example more than 90% of the beads, are in the range of from 0.5 millimeter to 1 millimeter, and independently thereof,

wherein the diameter of the particles is in the range of from 5 to 15 micrometer.

77. The method of any of claims 53 to 76, wherein the optically detectable label is selected from the group consisting of light reflecting particles, light absorbing particles, dyes, fluorescent particles, and autofluorescent particles.

78. The method of claim 53 comprising the further steps of

   i) comparing the Z-coordinates of different particles within each bead, and

   ii) selecting particles wherein the difference between Z-coordinates is less than a predetermined threshold value, delta-Z,

   iii) pairwise grouping the selected particles according to delta-Z values,

   iv) maintaining the X-coordinate and the Z-coordinate for each of the pairwise grouped particles, and

   v) switching the Y-coordinate between pairwise grouped particles, thereby obtaining an alternative set of 3D coordinates from which an alternative distance matrix can be calculated.

79. A method for identifying individual beaded polymer matrices in the composition according to any of claims 32 to 42, said method comprising the steps of

   i) determining the distance matrix for individual beads according to the method of any of claims 53 to 78,

   ii) deriving from each of the distance matrices generated in step i) all of the possible geometrical figures selected from triangles and

quadrangles, which can be generated by connecting particle co-ordinates with straight lines, and

iii) recording and storing the set of geometrical figures obtained in step ii) for each bead of the composition to be identified,

iv) selecting a subset of beads,

v) identifying one or more of the selected beads on the basis of a comparison of the set of possible geometrical figures selected from triangles and quadrangles of said bead(s) with all sets of possible geometrical figures selected from triangles and quadrangles recorded for the composition in step iii).

80. The method of claim 79, wherein the geometrical figures are triangles.

81. The method of any of claims 79 and 80, wherein each bead comprises 3 or 4 spatially immobilised particles.

82. The method of claim 79, wherein the geometrical figures are quadrangles.

83. A method for identifying at least one individually identifiable, spatially encoded, bead in a composition according to any of claims 32 to 42, comprising the steps of

i) determining the unique, spatial position of three or more particles in the at least one bead to be identified,

ii) deriving from the positions, a matrix of the distances between the three or more particles,

iii) deriving from the matrix, a set of all possible triangles defined by the three or more particles,

iv) identifying said at least one individually identifiable, spatially encoded bead based on comparison of the set of possible triangles with all sets of possible triangles capable of being stored for the composition of any of claims 32 to 58.

84. The method of any of claims 79 to 83 comprising the further step of performing a digital 3D registration of at least some of the identified beads, said 3D registration comprising generating a digital 3D reconstruction of the at least some identified beads based on the orthogonal pairs of images, and wherein the identification of individual beads is aided by an analysis of which of the generated 3D reconstructions represents the best fit to the original 3D registration.

85. A method for recording individual reaction steps involved in the step-wise synthesis of a chemical compound on a beaded polymer matrix according to any of claims 1 to 31, said method comprising the steps of

a) spatially immobilizing a plurality of particles in polymer beads or granulates,

b) isolating, preferably by automated selection, at least a subset of the spatially encoded beads or granulates provided in step a), and

c) recording and storing a distance matrix or a geometrical figure derivable from the distance matrix for each bead or granule, said distance matrix or geometrical figurde being preferably generated by the method of any of claims 53 to 78,

d) stepwise synthesising chemical compounds on functional groups of the encoded beads or granules, wherein the identy of each bead or granule is recorded and stored for each reaction step,

e) obtaining for each bead a record of individual reaction steps.

86. A method for identifying a chemical compound being synthesised on a beaded polymer matrix according to any of claims 1 to 31, said method comprising the steps of

a) performing the recording method of claim 85,
b) selecting beaded polymer matrices or granules of interest by using an assay or a diagnostic screen selective for the chemical compound having been synthesised on the beaded polymer matrix,
c) recording the distance matrix for each of the beaded polymer matrices selected in step b),
d) comparing the distance matrix recorded in step c) with all of the distance matrices recorded and stored in step c) of claim 85, thereby obtaining information about the identity of the selected bead,
e) identifying for each selected bead the sequence of individual steps having lead to the synthesis of the chemical compound, and
f) identifying, based the sequence of individual steps the chemical structure of the compound.

87. The method of claim 86, wherein the assay is a binding assay performed by measuring the binding of a protein to a ligand bound to the polymer matrix.

88. The method of claim 86, wherein the assay is performed by measuring an enzyme activity on a substrate bound to the polymer matrix.

89. The method of claim 86, wherein the assay is performed by measuring enzyme inhibition of a molecule bound to the polymer matrix.

90. The method of claim 86, wherein the assay is performed by measuring receptor interaction with a compound bound to the polymer matrix.

91. The method of any of claims 86 to 90, wherein said plurality of particles comprise a fluorescently detectable marker.

92. The method of claim 91, wherein said fluorescently detectable marker is a UV or visible light-excitable microspheres.

93. A device for recording and storing at least one image of at least one spatially encoded bead of the composition of different, spatially encoded beads according to any of claims 32 to 42, said device comprising

    i) at least one laser source of illumination,

    ii) a flow cell comprising an imaging section,

    iii) at least one pulse generator,

    iv) at least one image intensifier,

    v) at least one CCD camera, and

    vi) a computer running a program for calculation of distance matrices for individual spatially encoded beads,

    wherein the at least one CCD-camera for recording at least one fluorescence image of an individual encoded bead comprises a gated image intensifier for amplifying the fluorescence emission from the encoded bead,

    wherein each of said gated image intensifiers comprises an optical objective for focussing said imaging section of said flow cell onto the photosensitive area of each image intensifier, and

    wherein each optical objective comprises a fluorescence filter for blocking the light of said laser, said fluorescence filter being capable of transmitting the fluorescent emission from an individual encoded bead.

94. The device according to claim 93 further comprising a photo-sensor for detecting entry of an encoded bead into the imaging section of the flow cell.

95. The device according to claim 94, wherein the photo-sensor comprises an optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of said photo-sensor, and wherein said optical objective of said photo-sensor comprises a fluorescence filter for blocking the light of said laser, and the fluorescence filter is capable of transmitting the fluorescence emission from an individual encoded bead.

96. The device according to any of claims 94 and 95, wherein the laser source of illumination is a continuous wave laser.

97. The device according to any of claims 94 to 96, wherein the photo-sensor comprises an optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of said photo-sensor, and wherein said optical objective of said photo-sensor comprises a fluorescence filter for blocking the light of said laser, and wherein said fluorescence filter is capable of transmitting the fluorescence emission from an individual encoded bead.

98. The device according to any of claims 94 to 97, wherein the device comprises two or more CCD-cameras for recording at least one fluorescence image of an individual encoded bead, wherein each of said two or more CCD-cameras comprises a gated image intensifier for amplifying the fluorescence emission from the encoded bead, wherein each of said gated image intensifiers comprises an optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of each image intensifier, wherein each optical objective comprises a fluorescence filter for blocking the light of said laser, and wherein the fluorescence filter is capable of transmitting the fluorescence emission from an individual encoded bead.

99. The device according to any of claims 94 to 98, wherein the pulse generator is an electrical square wave pulse generator for triggering said two or more CCD-cameras and/or said two or more image intensifiers.

100. The device according to any of claims 94 to 99 further comprising an image storage system comprising

a) a framegrabber for recording the images from said two or more CCD-cameras,

b) an electronic memory-device for storing said images from said frame-grabber,

c) a program code for controlling said electronic memory-device, and

d) a computer for integrating said framegrabber and said electronic memory device and for executing said program code.

101. The device according to any of claims 94 to 100 further comprising a fast computer for on-line image processing of the images and determination of the number of particles per encoded bead and the set of possible distance matrices.

102. The device according to claim 101 further comprising a switching valve positioned downstream from the flow cell and means for controlling said valve on the basis of the number of particles per encoded bead.